[0085] $$\ln(R_{tot})_1 = C_1 - 2\{ I_D[HbO]\varepsilon_{HbO1} + I_D[Hb]\varepsilon_{Hb1}\}$$

$$\ln(R_{tot})_2 = C_2 - 2\{ I_D[HbO]\varepsilon_{HbO2} + I_D[Hb]\varepsilon_{Hb2}\}$$

(8) $$\ln(R_{tot})_1 - \ln(R_{tot})_2 = C_1 - C_2 - 2\, I_D \{ [HbO](\varepsilon_{HbO1} - \varepsilon_{HbO2}) + [Hb](\varepsilon_{Hb1} - \varepsilon_{Hb2})\}$$

[0086] Thus, if $(\varepsilon HbO_1 - \varepsilon HbO_2) > 0$ and $(\varepsilon Hb_1 - \varepsilon Hb_2) < 0$, then $\ln(Rtot)_1 - \ln(Rtot)_2$ increases as [HbO] increases or [Hb] decreases. For example, if the extinction coefficient of HbO is greater at wavelength 1 than wavelength 2, and Hb has an extinction coefficient that is less at wavelength 1 than wavelength 2, then as the difference between the signals (i.e., the difference between wavelength 1 – wavelength 2) increases, the ratio of HbO to Hb will increase. In many embodiments, this method of characterizing total hemoglobin concentration is performed first, such that this method of characterizing HbO/Hb ratios is performed on a site having a high hemoglobin concentration. In other words, because the total hemoglobin concentration affects the difference calculation, characterizing HbO/Hb ratios should be performed on a site having a substantially high total hemoglobin concentration.

[0087] Specifically, a potential site is illuminated with two wavelengths from two light sources, where such light sources may include one or more LED, one or more laser diode, etc. The wavelengths are chosen such that the molar extinction coefficient deltas of HbO and Hb are different between the two wavelengths, i.e., as one goes up the other goes down, as described above. At least one photodetector detects the signal from the site, i.e., the absorbance of the light, where such signal is related to an HbO/Hb ratio, according to the above described equations. The site is then further characterized as being appropriate or not for a particular test. Site appropriateness will be described in greater detail below.

III. Determining the Appropriateness of a Site for a Particular Test

[0088] As mentioned above, the appropriateness of a site for a particular test is determined by the subject methods. Referring now to steps 3, 4 and 7 of Figure 1, as described above, once a site is characterized by flow and/or sample type, its appropriateness in regards to the particular test to be performed is evaluated. Such appropriateness is best described in reference to Figure 2, which shows certain sample

test parameters and their correlation to particular samples obtainable from a site. For example, certain tests require a minimum sample volume. Thus, a site which is characterized as being capable of producing or expressing a greater volume of sample (a site having higher flow rate) would be preferable to a site not so capable, e.g., high flow of arterial/capillary and/or venous would be more appropriate versus a low flow site of arterial/capillary and/or venous, unless the particular test required interstitial fluid as opposed to arterial/capillary or venous blood. As such, test results meeting the requirements of such samples would be determined to be appropriate.

[0089] Also, certain tests such as glucose tests calibrated to whole blood may require a certain type of sample such as blood, blood constituents or the like as the appropriate fluid sample and as such a site will be determined appropriate for such a test if the site is characterized as arterial/capillary and/or venous and likewise inappropriate if it is characterized as having interstitial fluid. However, certain other tests such as glucose tests calibrated to interstitial fluid may, accordingly, require interstitial fluid as the appropriate fluid sample and as such a site will be determined appropriate if the site is characterized as having interstitial fluid and likewise inappropriate if it does not.

[0090] Furthermore, some tests may require arterial blood instead of venous blood, or *vice versa*, and as such will be determined appropriate if the site is characterized as having the requisite arterial or venous blood and inappropriate if it does not. In other words, a test that requires arterial/capillary and/or venous blood would thus correlate to a high flow arterial/capillary and/or high flow venous site. A test that requires interstitial fluid would thus correlate to a low flow interstitial fluid site. A site characterized as low flow arterial/capillary or venous site would thus likely not be appropriate for any test.

[0091] As described above, in many embodiments of the subject methods, appropriateness of a site for a particular test is typically accomplished automatically by a microprocessor, where the microprocessor works under the control of a software program and includes all the code necessary for it to carry out the steps required to determine if a site is appropriate for a particular test.

IV. Skin Piercing

[0092] Once an appropriate site has been determined, sample is then accessed and collected (steps 8 and 9 of Figure 1). Typically, sample is collected from the dermis and

epidermis. In certain methods, the sampling site may be stimulated to increase the volume and/or rate of sample produced or expressed at the sampling site.

[0093] Accordingly, in some embodiments, at least one skin-piercing element is inserted into the skin of a patient or user of the subject invention to access physiological fluid. Depending on the type of physiological sample to be obtained, the at least one skin-piercing element may penetrate to a particular skin layer, such as the dermis and epidermis layers. Typically, the at least one skin-piercing element is inserted into the skin for about 0.0001 to 60 seconds, usually about 0.0005 to 30 seconds and more usually from about 0.001 to 15 seconds so as to ensure an adequate sampling volume of the targeted physiological fluid is obtained.

[0094] The at least one skin-piercing element may be activated manually by the user by releasing an actuating element associated with the at least one skin-piercing element, *e.g.*, by depressing a button or the like on a device which activates the spring-loaded element towards the skin, or may be automatically activated to pierce the skin, for example triggered automatically when a suitable sampling site is located.

[0095] In certain embodiments of the subject methods, the at least one skin-piercing element, or one or more elements operatively associated therewith, stimulates the site to produce or express a greater volume and/or rate of the physiological fluid desired of the physiological fluid desired, *i.e.*, increases the rate of expression of physiological fluid. For example a fluid enhancing element, *e.g.*, an ultrasonic element or the like, may be used to create vibrations at the site during fluid access and collection, where such vibrations stimulate fluid expression. In certain embodiments, the fluid enhancing means may include, in addition to or in place of other fluid stimulating elements, a temperature element to increase the temperature of the site to stimulate fluid expression. The fluid enhancing element may be operatively associated with the at least one skin-piercing element such that the at least one skin-piercing element stimulates fluid expression itself while it accesses the fluid from the site. In any event, in those embodiments employing an ultrasonic element to stimulate sample expression from a site, such an ultrasonic element typically vibrates at a frequency in the range from about 10 to 1000 Hz, where such vibrations stimulate the expression of physiological fluid, e.g., increase the volume and/or rate of sample production.